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Remarks

Applicants thank the examiner for the courtesy of a telephone conference on March 31, 2004 to discuss the rejection based on 35 U.S.C. §112. In light of the discussion, claims 1, 2, 4 and 5 have been amended. No new subject matter is believed to have been added.

To assist the Examiner, applicants would like to point out that an endonuclease is so named because of the particular organism from which it is first isolated and hence is endogenous to. For example, MseI is endogenous to a particular *Micrococcus species* strain (NEB446, also known as ATCC No. PTA-2421). MnII is endogenous to a particular *Moraxella nonliquefaciens*, MlyI is endogenous to a particular *Micrococcus lylae*. NEB catalog 2002-2003 pg 46 and 47 is attached hereto to illustrate this. It can be seen from these two pages that 3 different *Micrococcus* restriction endonucleases are described from different strains each with a different cleavage site and encoded by different DNA sequences. Of these endonucleases, only the DNA encoding MseI is obtainable from ATCC PTA-2421.

Objection under 37CFR § 1.83(a)

The Examiner has queried Figure 1 with respect to the Brief Description of Figure 1B. The Examiner has further objected to the absence of "A" and "B" on Figure 1.

This description provides verification of the cleavage activity of MseI on DNA from cells transformed with the M.MseI (pVR-18). The results of this experiment are reported in detail in Example IV and

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present Figure 1. Consequently, applicants have deleted the description to Figure 1b and modified the text on page 16 to remove the "A". With the present modifications, no further alteration to the Figure 1 itself is necessary.

Rejection under 35U.S.C. 6112 first paragraph

The Examiner objects to claim 1 prior to amendment because of the reliance on a specified Micrococcus species. Consequently, applicants have amended claim 1 to require that the DNA be obtainable from the ATCC deposited strain (PTA-2421) which is a recombinant *E.coli* containing the DNA encoding the restriction endonuclease produced by the specified Micrococcus strain.

In the telephone conference with the Examiner on March 31, 2004 , the language "obtainable from" as applied to a DNA encoding a restriction endonuclease was discussed. It was agreed that the term "obtainable" is descriptive when used in the context of a recombinant DNA in a deposited recombinant clone of *E.coli* wherein the specification further provides sequences for the recombinant DNA encoding the claimed endonuclease and methylase.

The amendment in claim 1 in which the DNA encoding the specified restriction endonuclease is obtainable from a deposited recombinant *E.coli* is supported by an adequate description in which the DNA sequence is included in the specification. The significant feature of the invention as claimed is a novel and non-obvious DNA which is characterized very specifically by a sequence and by a deposited recombinant *E.coli*. The possibility that the isolated DNA

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may be derived from native sources in addition to those specified does not detract from the description of the isolated DNA molecule.

Any person of ordinary skill in the art can obtain the DNA from the deposited strain or related isolate in light of the description in the specification without any undue experimentation. Moreover, anyone of ordinary skill in the art based on the description in the specification would be able to use the DNA sequence disclosed in the present application to conduct a GenBank search and to identify the isolated DNA in its native context.

The Examiner has rejected claims 3-6 because of the missing statement in the deposit requirements. Applicants have amended the specification on Page 61 to state (1) that the deposited material will be available to the public on issuance of the patent and (2) the address of the depository.

For the reasons set forth above, Applicants respectfully submit that the rejections set forth in the Official Action of October 30, 2003 have been overcome and that this case is in condition for immediate allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Applicants petition for a three-month extension of time in which to file this response. Please charge deposit account no. 14-0740 in the amount of \$475. Applicants authorize that any additional fees that may be due be charged against this account number.

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Should the Examiner wish to discuss any of the amendments and/or remarks made herein, the undersigned Attorney would appreciate the opportunity to do so.

Respectfully submitted,

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ORDERS

Restriction Endonucleases

Mbo I

#R0147S 500 units \$60
 #R0147L 2,500 units \$240
 for high (DD) concentration, order #R0147M (2,500 units)

5'...**G A T C**... 3'
 3'...**C T A G**... 5'

Source: An *E. coli* strain that carries the cloned Mbo I gene from *Moraxella bovis* (ATCC 10900)

Reaction Conditions: NEBuffer 3
 100 mM NaCl, 50 mM Tris-HCl, 10 mM MgCl₂, 1 mM dithiothreitol (pH 7.9 @ 25°C). Incubate at 37°C.

Ligation and Recutting: After 100-fold overdigestion with Mbo I, > 95% of the DNA fragments can be ligated and recut.

Concentration: 5,000 and 25,000 units/ml.
 Assayed on λ DNA (*dam*⁻).

Storage Conditions:

50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl₂, 1 mM BSA and 50% glycerol. Store at -20°C.

Diluent Compatibility:**Heat Inactivation:**

Note: Dpn II and Sau3A I cleave at the same site. Mbo I cleaves to leave a 5' single-base extension which can be efficiently ligated into Blunt-ended fragments. Dpn II or Sau3A I cleaved DNA is blocked by *dam* methylation. Isoschizomer Sau3A I is not sensitive to genomic DNA is Impaired by *dcm* methylation (see p. 252).

Blocked by *dam* methylation. Isoschizomer Sau3A I is not sensitive to genomic DNA is Impaired by *dcm* methylation (see p. 252).

Mbo II

#R0148S 250 units \$55
 #R0148L 1,250 units \$220

5'...**G A A G A (N)₈**... 3'
 3'...**C T T C T (N)₇A**... 5'

Source: An *E. coli* strain that carries the cloned Mbo II gene from *Moraxella bovis* (ATCC 10900)

Reaction Conditions: NEBuffer 2
 50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl₂, 1 mM dithiothreitol (pH 7.9 @ 25°C). Incubate at 37°C.

Ligation and Recutting: After 10-fold overdigestion with Mbo II, approximately 50% of the DNA fragments can be ligated. Of these, > 95% can be recut.

Concentration: 5,000 units/ml.
 Assayed on λ DNA (*dam*⁻).

Storage Conditions:

50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl₂, 1 mM BSA and 50% glycerol. Store at -20°C.

Diluent Compatibility:**Heat Inactivation:**

Note: Mbo II produces DNA fragments with a single-base 3' extension which are more easily ligated than blunt-ended fragments. Efficient ligation can be achieved by using NEBuffer 2 (NEB #M2200).

Blocked by overlapping *dam* methylation.

Incubations longer than 1 hour are not recommended.

Mfe I

#R0589S 500 units \$60
 #R0589L 2,500 units \$240

5'...**C A A T T G**... 3'
 3'...**G T T A A C**... 5'

Source: An *E. coli* strain that carries the cloned Mfe I gene from *Mycoplasma fermentans* (N.F. Halden)

Reaction Conditions: NEBuffer 4
 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol (pH 7.9 @ 25°C). Incubate at 37°C.

Ligation and Recutting: After 20-fold overdigestion with Mfe I, > 95% of the DNA fragments can be ligated and recut.

Concentration: 10,000 units/ml. Assayed on λ DNA.

Storage Conditions: 50 mM NaCl, 10 mM Tris-HCl, 10 mM potassium acetate, 10 mM magnesium acetate, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol. Store at -20°C. Incubate at 37°C. Incubate at 37°C.

Diluent Compatibility:**Heat Inactivation:**

Note: Mun I is an Isoschizomer of Mfe I.

Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Concentration: 3,000 units/ml. Assayed on λ DNA.

Mlu I

#R0198S 1,000 units \$55
 #R0198L 5,000 units \$220

5'...**A C G C G T**... 3'
 3'...**T G C G C A**... 5'

Source: *Micrococcus luteus* (IFO 12992)

Reaction Conditions: NEBuffer 3
 100 mM NaCl, 50 mM Tris-HCl, 10 mM MgCl₂, 1 mM dithiothreitol (pH 7.9 @ 25°C). Incubate at 37°C.

Ligation and Recutting: After 10-fold overdigestion with Mlu I, > 95% of the DNA fragments can be ligated and recut.

Concentration: 10,000 units/ml. Assayed on λ DNA.

Storage Conditions: 100 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol. Store at -20°C.

Diluent Compatibility:**Heat Inactivation:**

Note: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 252).

Source: An *E. coli* strain that carries the cloned Mlu I gene from *Micrococcus luteus* (IFO 12992)

Reaction Conditions: 50 mM NaCl, 10 mM Tris-HCl, 10 mM dithiothreitol (pH 7.1), 100 µg/ml BSA. Incubate at 37°C.

Ligation and Recutting: After 10-fold overdigestion with Mse I, > 95% of the DNA fragments can be ligated and recut.

BSA: Requires BSA

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Source: An *E. coli* strain that carries the cloned *Mly I* gene from *Micrococcus luteus* (NBL 2048)

Reaction Conditions: NEBuffer 4 + BSA

50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol (pH 7.9 @ 25°C). Supplement with 100 µg/ml BSA. Incubate at 37°C.

Ligation and Recutting: After 20-fold overdigestion with *Mly I*, approximately 75% of the DNA fragments can be ligated and recut.

Concentration: 10,000 units/ml.

Assayed on λ DNA.

Storage Conditions: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol. Store at -20°C.

#R0610S 1,000 units \$55
#R0610L 5,000 units \$220

Diluent Compatibility: Diluent A, see p. 237.

Heat Inactivation: 65°C for 20 minutes.

Note: *Mly I* is an isoschizomer of *Pst I* that generates blunt-ended DNA fragments.

Not sensitive to *dcm*, *dcm* or mammalian CpG methylation.

5'...GAGTC(N)₅...3'
3'...CTCA G(N)₅...5'

Source: An *E. coli* strain that carries the cloned *Mnl I* gene from *Moraxella nonliquefaciens* (ATCC 17953)

Reaction Conditions: NEBuffer 2 + BSA

50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl₂, 1 mM dithiothreitol (pH 7.9 @ 25°C). Supplement with 100 µg/ml BSA. Incubate at 37°C.

Ligation and Recutting: After 2-fold overdigestion with *Mnl I*, approximately 50% of the DNA fragments can be ligated. Of these, > 95% can be recut.

Concentration: 5,000 units/ml.

Assayed on λ DNA.

Storage Conditions: 200 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 500 µg/ml BSA and 50% glycerol. Store at -20°C.

#R0163S 250 units \$55
#R0163L 1,250 units \$220

Diluent Compatibility: Diluent B, see p. 237.

Heat Inactivation: 65°C for 20 minutes.

Note: *Mnl I* produces DNA fragments that have a single-base 3' extension which are more difficult to ligate than blunt-ended fragments. More efficient ligation can be achieved by using the Quick Ligation Kit (NEB #M2200).

Not sensitive to *dcm*, *dcm* or mammalian CpG methylation.

5'...CCTC(N)₇...3'
3'...GGAG(N)₆...5'

Source: An *E. coli* strain that carries the cloned *Msc I* gene from *Micrococcus* species (C. Polisson)

Reaction Conditions: NEBuffer 4

50 mM potassium acetate, 20 mM Tris acetate, 10 mM magnesium acetate, 1 mM dithiothreitol (pH 7.9 @ 25°C). Incubate at 37°C.

Ligation and Recutting: After 20-fold overdigestion with *Msc I*, > 95% of the DNA fragments can be ligated and recut.

Concentration: 3,000 and 15,000 units/ml.

Assayed on λ DNA.

Storage Conditions: 150 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol. Store at -20°C.

#R0534S 250 units \$55
#R0534L 1,250 units \$220
For high (5X) concentration, order #R0534M (1,250 units)

Diluent Compatibility: Diluent B, see p. 237.

Heat Inactivation: 65°C for 20 minutes.

Note: *Msc I* is an isoschizomer *Bal I*.

Blocked by overlapping *dcm* methylation (see p. 253). The single *Msc I* site in pBR322 overlaps a *dcm* methylation site; consequently, pBR322 which has been grown in a *dcm*⁻ host should be used for cloning.

5'...TGGCCA...3'
3'...ACC₆GTT...5'

Source: An *E. coli* strain that carries the cloned *Mse I* gene from *Micrococcus* species (R. Morgan)

Reaction Conditions: NEBuffer 2 + BSA

50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl₂, 1 mM dithiothreitol (pH 7.9 @ 25°C). Supplement with 100 µg/ml BSA. Incubate at 37°C.

Ligation and Recutting: After 5-fold overdigestion with *Mse I*, > 95% of the DNA fragments can be ligated and recut.

Concentration: 4,000 and 20,000 units/ml.
Assayed on λ DNA.

#R0525S 500 units \$55
#R0525L 2,500 units \$220
For high (5X) concentration, order #R0525M (2,500 units)

Storage Conditions: 50 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol. Store at -20°C.

Diluent Compatibility: Diluent A, see p. 237.

Heat Inactivation: 65°C for 20 minutes.

Note: Not sensitive to *dcm*, *dcm* or mammalian CpG methylation.

5'...T₇TAA...3'
3'...AAT₄T...5'

Requires BSA

Methylation Sensitivity

Heat Inactivation

Blue/White Colonies